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Environmental Transformation Products of Nitroaromatics and Nitramines

Literature Review and Recommendations for Analytical Method Development

Marianne E. Walsh

February 1990

For conversion of SI metric units to U.S./British customary units of measurement consult ASTM Standard E380, Metric Practice Guide, *published by the American Society for Testing and Materials, 1916 Race St., Philadelphia, Pa. 19103.*

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U.S. Army Corps of Engineers
Cold Regions Research & Engineering Laboratory

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Prepared for
U.S. ARMY TOXIC AND HAZARDOUS MATERIALS AGENCY
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PREFACE

This report was prepared by Marianne E. Walsh, Research Physical Scientist, Applied Research Branch, Experimental Engineering Division, U.S. Army Cold Regions Research and Engineering Laboratory. Funding for this project was provided by the U.S. Army Toxic and Hazardous Materials Agency, Aberdeen Proving Ground, Maryland (R-90 Multi-Analytical Services), Martin H. Stutz, Project Monitor.

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ABBREVIATIONS

2-A	2-amino-4,6-dinitrotoluene
4-A	4-amino-2,6-dinitrotoluene
2,4-DA	2,4-diamino-6-nitrotoluene
2,6-DA	2,6-diamino-4-nitrotoluene
2-OHA	2-hydroxylamino-4,6-dinitrotoluene
4-OHA	4-hydroxylamino-2,6-dintrotolune
4,4'-Az	2,2',6,6'-tetranitro-4,4'-azoxytoluene
2,2'-Az	4,4',6,6'-tetranitro-2,2'-azoxytoluene
2,4'-Az	2',4,6,6-tetranitro-2,4'-azoxytoluene
TNB	trinitrobenzene
FAD	flavin adenine dinucleotide
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide, reduced form
DNT	dinitrotoluene
DNB	1,3-dintrobenzene
TNT	1,3,5-trinitrotoluene
THB	trihydroxybenzene
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
RDX	hexahydro-1,3,5-trinitro-1,3,5-triazine
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
DNX	hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
AcHMX	octahydro-1-(N)-acetyl-3,5,7-trinitro-1,3,5,7-tetrazocine (SEX)
AcRDX	hexahydro-1-(N)-acetyl-3,5-dinitro-1,3,5-triazine (TAX)
GC/MS	gas chromatography/mass spectrometry

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INTRODUCTION

One of the Army's major environmental problems is the contamination of soil and water with residues of explosives at many military facilities. This contamination occurred during the disposal of wastewater from production and load-and-pack operations, burning or detonation of off-specification material, and destruction of out of date explosives. To monitor these sites, analytical methods were required to detect and quantify these military-unique compounds in environmental matrices. Under the auspices of U.S. Army Toxic and Hazardous Materials Agency (THAMA), CRREL has developed methods for the determination of explosives in environmental samples. Since some of the transformation products of these explosives may be of environmental concern, it may also be necessary to develop analytical methods for some of these byproducts. The objective of this study was to review the literature to identify the products formed after explosives enter the environment and to identify those compounds for which certified analytical methods should be developed. Also, analytical methodology already developed for these compounds is summarized.

NITROAROMATICS

2,4,6-Trinitrotoluene (TNT)

The microbial transformation of 2,4,6-trinitrotoluene (TNT) has been studied extensively. Two literature reviews concerning the environmental fate of TNT were published in 1980. Spanggord et al. (1980a) compiled the literature on the transport and transformation products of TNT and ten other munition wastewater constituents in two rivers and a bay. Isbister et al. (1980) reviewed studies on microbial degradation pathways of TNT, 2,4-DNT and RDX. The conclusions of both reports were that the TNT molecule is transformed by reduction of the nitro groups to form monoaminodinitrotoluenes (2-A, 4-A) and condensation products such as tetranitroazoxytoluene. Nitroreduction is accomplished by various microorganisms including bacteria, yeast and fungi. No definitive evidence of ring cleavage was found. Transformation products reported in the literature prior to 1980 are listed in Table 1.

Reports published since 1980 describe the extensive work on composting by researchers at the U.S. Army Natick Research and Development Laboratory (Kaplan and Kaplan 1982a,b,c) and at the Atlantic Research Corporation (Isbister et al. 1980, 1984). Information on microbiological degradation products has also been published in the Russian, Chinese and Indian literature.

The biotransformation pathway for TNT in simulated composting systems proposed by Kaplan and Kaplan (1982a) is shown in Figure 1. The compost was incubated with uniformly labeled ^{14}C-TNT for 91 days. After 24 days, TNT, 2-A and 4-A were identified in the test compost, with 4-A as the predominant amine. After 91 days, TNT, 2-A, 2,4-DA, 2,6-DA, 4,4'-Az and 2,4'-Az were extracted. No significant $^{14}CO_2$ was recovered. However, with time, a progressively greater percentage of ^{14}C-labeled material appeared to bind to the organic matter fractions of the compost. The authors (Kaplan and Kaplan 1982b) caution that the "mere disappearance of a compound under composting conditions is not sufficient evidence that the resulting environmental hazard has been effectively eliminated." They (Kaplan and Kaplan 1983) found similar binding reactions between TNT and its metabolites and humic material in

Table 1. Summary of degradation products of TNT reported prior to 1980.

	Media	*End products*
Jerger and Chynoweth (1966)	Anaerobic digesters	4-OHA
Enzinger (1970)	Broth cultures	Probably 4-A and 4,4'-Az
Klausmeier et al. (1973)	Fungi, yeasts, actinomycetes, gram-positive	Reduction products
Won et al. (1974)	Enrichment cultures with *Pseudomonas*-like organisms	Azoxytoluenes, 2-A, 4-OHA, DA's
Traxler et al. (1974)	Gram-negative bacteria, mineral salt basic media	Some CO_2
McCormick et al. (1976)	Aerobic and anaerobic cond. (enzymes from *Veillonella alkalescens*)	Arylamines
Jerger et al. (1976)	Broth cultures	4-OHA, 2-OHA, 4-A, 2-A, 4,4'-Az, 2,4-DA
Parrish (1977)	Cultures of fungi	4-A, 4-OHA, 4,4'-Az
Amerkhanova and Naumova (1978)	*Ps. denitrificans* cultured on basic synthetic media	2-A and 4-A
Carpenter et al. (1978)	Activated sludge	4-A, 2-A, 2,4-DA, 2,6-DA, polyamide macromolecules
Hoffsommer et al. (1978)	Activated sludge with supplemental nutrients	Amines and complex polar products
Osmon and Andrews (1978)	Soil amended with nutrients	4-A
Pereira et al. (1979)	Groundwater	2-A, 4-A
Burlinson (1980)	Aqueous systems: Sunlight	1,3,5-TNB, 2,4,6-trinitrobenzaldehyde, trace 2-A and 4-A, 3,5-dinitroaniline
	Dark	Known biotransformation products

soil. Hoffsommer et al. (1978) also reported the presence, in decreasing order, of 4-A, 2-A, 2,4-DA and 2,6-DA when 10–50 mg/L of aqueous TNT was pumped into an aerated oxidation ditch facility containing bacterial floc from activated sludge microorganisms and supplemental nutrients. The amino compounds accounted for 12% of the continuously fed TNT, and the relative amounts on a weight basis were 4-A:2-A:2,4-DA:2,6-DA, 1.0/0.12/0.10/0.013. Hoffsommer et al. (1978) also did bioconversion experiments using uniformly labeled TNT. After 3 days, 34% of the total ^{14}C

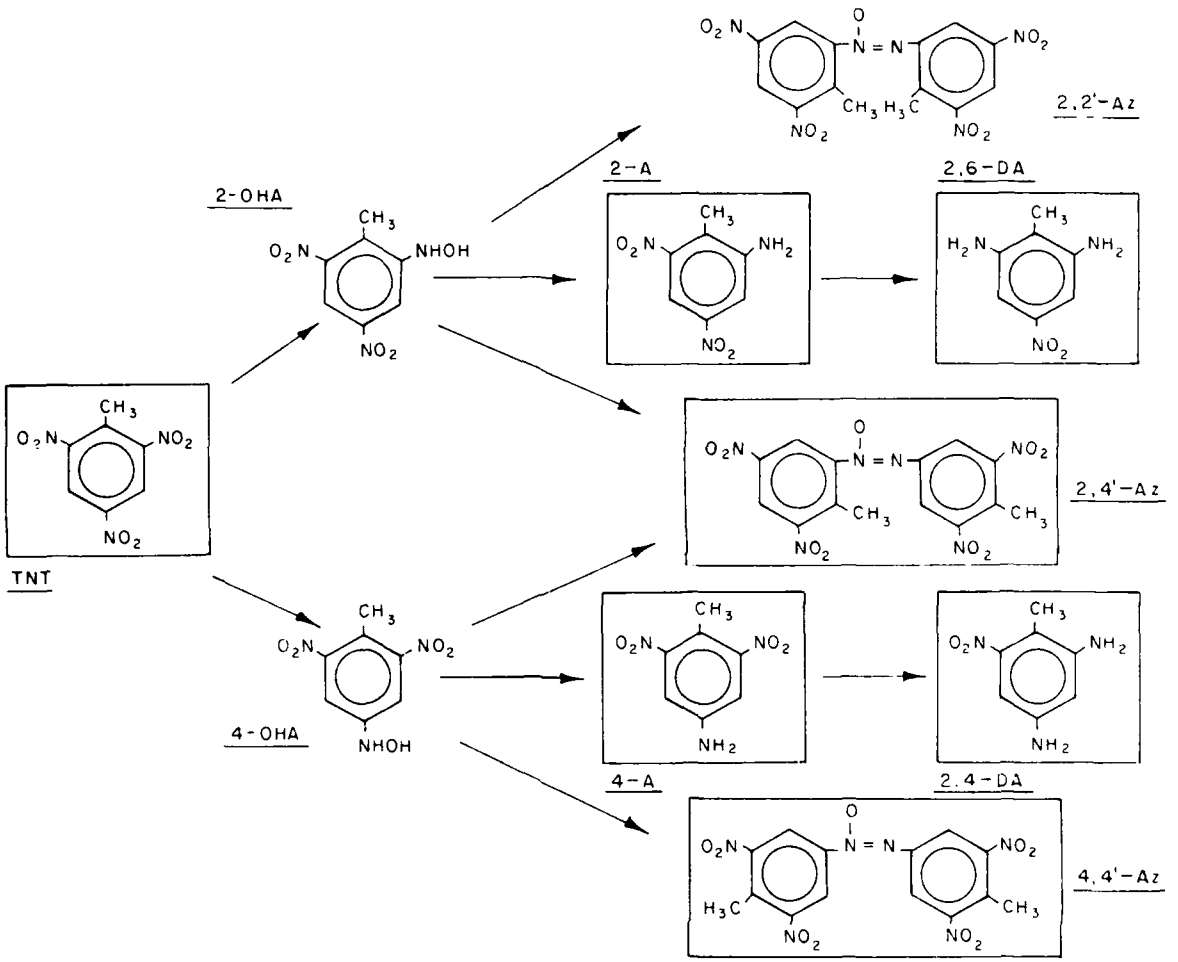

Figure 1. Pathway for TNT (after Kaplan and Kaplan 1982a).

activity was accounted for by the amines (26% in the supernate and 8% in the floc). After 30 days, the activity associated with the amines had decreased to only 3% (less than 0.28% in the supernate and 2.8% in the floc). The largest portion of the ^{14}C activity (40–64%) was associated with the floc. The authors postulated that this material was composed of water-insoluble polymeric-like substances having a high molecular weight.

Isbister et al. (1984) used uniformly ring-labeled ^{14}C-explosives in a laboratory-scale composting study. The reduction of TNT levels in the compost correlated with a significant increase in the compost-bound ^{14}C activity. TNT reduction products (2-A, 4-A, 2,6-DA or 2,2'-Az) were not identified in the 3-week composts and only minor amounts were determined in the 6-week composts. The authors conclude that TNT is transformed into insoluble macromolecules that behave like humus, and that composting may be used to immobilize high concentrations of explosives in sediments and soils. No evidence of ring cleavage was found by Kaplan and Kaplan or Isbister et al.

To study the degradation of TNT, RDX, HMX and 2,4-DNT in soil, Greene et al. (1985) applied simulated pink water to a series of soil columns that varied in carbon supplementation and pink water flow rate. The experimental columns were inoculated with organisms from an activated sludge, from an anaerobic sludge digest and from garden soil. One column was not inoculated and was sterilized with 0.5% mercuric chloride. The columns were irrigated continuously for 110 days, and the influent and leachate were sampled weekly. For all columns, including the sterile column, the amount of TNT recovered was low (0–24%), indicating that the TNT remained in the soil column or was transformed. Analysis of the leachate from

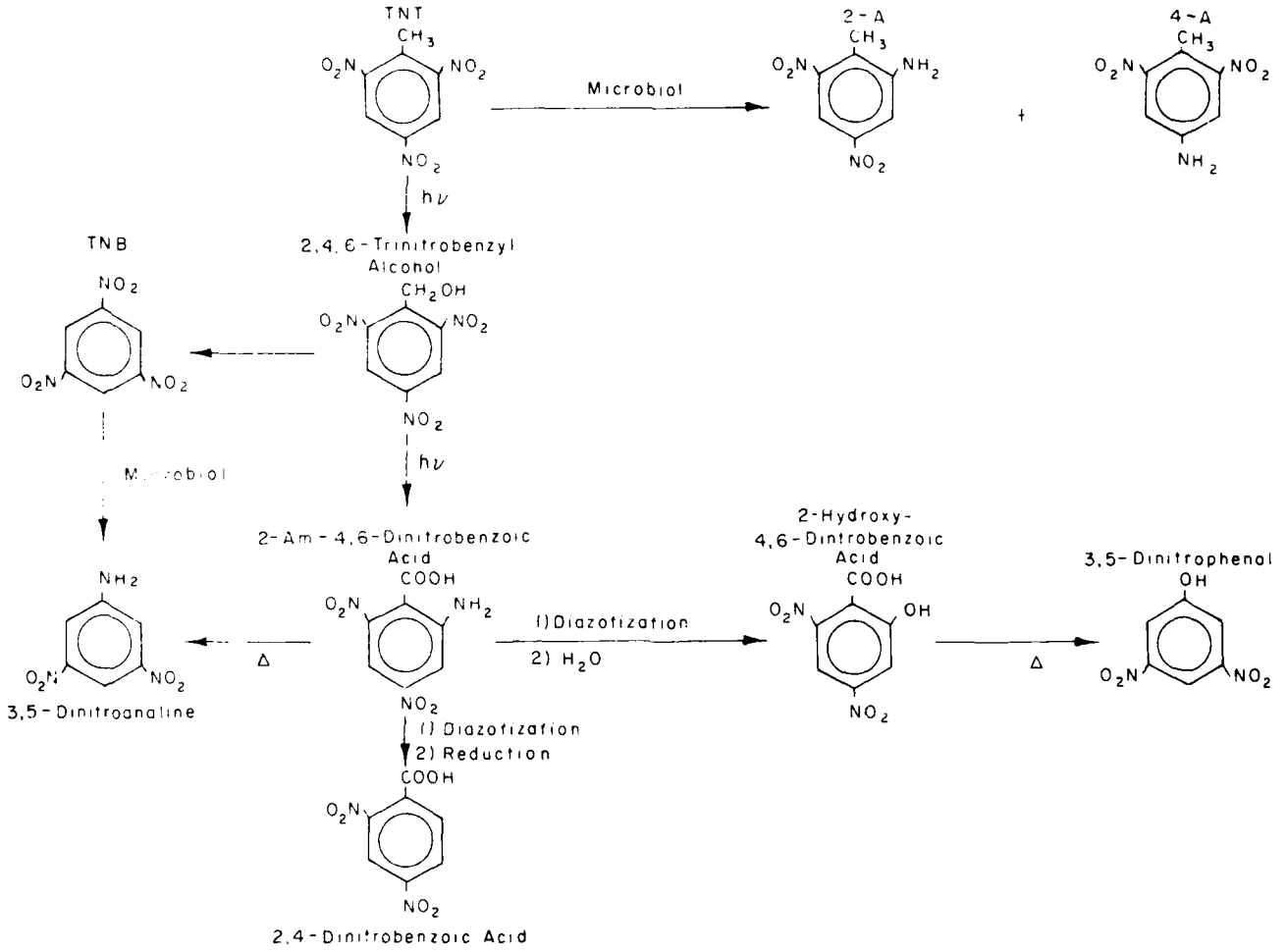

Figure 2. Pathway to 2-amino-4,6-dinitrobenzoic acid (after Spanggord 1983a).

the columns with fast flow (100 mL/day), with and without a carbon supplement (2.0 g/L glucose), revealed the presence of 4-A. Also, 2-A was detected in the leachate from the column with fast flow and carbon supplementation.

Spanggord et al. (1980b), while studying the environmental fate of TNT in selected water bodies, identified the monoaminodinitrotoluenes (2-A and 4-A), diaminonitrotoluenes (2,4-DA and 2,6-DA), and tetranitroazoxytoluene (4,4'-Az) derivatives of TNT as metabolites. In addition, an intermediate metabolite was found that contained a thio-methyl group, indicating that a sulfur compound may play a role in the reduction of nitro groups. Equal amounts of 2-A and 4-A were formed. They did not detect ring cleavage.

Spanggord et al. (1980b) also reported TNT photoproducts. Less than 20% of the TNT degraded could be accounted for by single aromatic ring compounds such as 1,3,5-TNB, 4,6-dinitroanthranil, 2,4,6-trinitrobenzaldehyde, 2,4,6-trinitrobenzonitrile and 2,4,6-trinitrobenzoic acid. Another 20% of the TNT is transformed to nitroazoxy derivatives formed by the coupling of nitroso and hydroxylamine products. While Spanggord and co-workers identified most of the above products when more than 50% of the available TNT was photolyzed, only one product was

detected when UV exposure was so short that only 10% of the TNT was degraded. This unidentified compound formed a pink solution when collected from an HPLC column in 30/70 acetonitrile:water and reverted thermally to TNT. Properties of this compound, such as its water solubility, NMR spectrum and low volatility, implied a TNT anion-type structure.

Spanggord et al. (1983a) investigated the persistence of TNT and RDX in waste disposal lagoons at the Louisiana Army Ammunition plant. TNT was transformed by both photochemical and microbiological processes. The major biotransformation products were identified as 2-A and 4-A, while a major stable phototransformation product was 2-amino-4,6-dinitrobenzoic acid (Fig. 2).

Kearney et al. (1983) examined whether ultraviolet-ozonation (UV-O_3) of aqueous ^{14}C-TNT (uniformly labeled) facilitated subsequent TNT metabolism by soil microorganisms. The products of UV-O_3 were 1,3,5-TNB, 2,4,6-trinitrobenzaldehyde, 3,5-dinitrophenol, 3,5-dinitrocatechol, 3,5-dinitrohydroquinone and oxalic acid. There was substantial binding of TNT and its bioconversion products to soil. No evidence of ring cleavage was found. While some metabolic $^{14}CO_2$ evolution was measured, it was attributed to the methyl carbon of TNT.

Kanekar and Godbole (1984) isolated microorganisms from soil samples collected along the banks of the Mula River, India, opposite the wastewater discharge point of a TNT manufacturing facility. The most numerous genera present was *Pseudomonas*, and among these a new species was identified, *Pseudomonas trinitrotoluenophila*. In a synthetic medium having TNT as the sole source of carbon and nitrogen, this microorganism gave 100% TNT degradation after 30 days of incubation. The authors detected CO_2 as one of the products of microbial degradation and claim that cleavage of the benzene ring may have occurred, although it is possible that all the carbon was provided by the methyl group, not the benzene ring, of TNT. No attempts to identify other degradation products were reported and ^{14}C-labeled TNT was not used.

The microbial degradation of TNT has been studied at Kazan State University, USSR, by Naumova, Selivanovskaya and co-workers. While the papers describing this work are published in Russian, the abstracts are available in English. The following is a summary of the information gleaned from the abstracts.

Naumova et al. (1982) studied the metabolism of TNT by several yeasts and fungi. Most of the TNT was reduced to 2-A and 4-A, with 2-A prevailing. Naumova et al. (1983) found the amount of 2-A was 2–3 times greater than the 4-A when TNT was transformed by *Pseudomonas denitrificans* and *Escherichia coli*. Nitroreduction was stimulated by FAD, Mg and Mn, and occurred at a pH range of 5.5–7.8. Reduction was dependent on NAD and NADH.

Selivanovskaya et al. (1986) studied the degradation of 2,4-DA, which was produced by microbial catabolism of TNT. They proposed the following pathway: 2,4-DA → phloroglucinol carboxylic acid → phloroglucinol (1,3,5-THB) → pyrogallol (1,2,3-THB) → ring cleavage (Fig. 3).

Sleivanovskaya et al. (1987) found that aerobic biodegradation of TNT by *Pseudomonas fluorescens* was expedited by the addition of clay. The bacte-

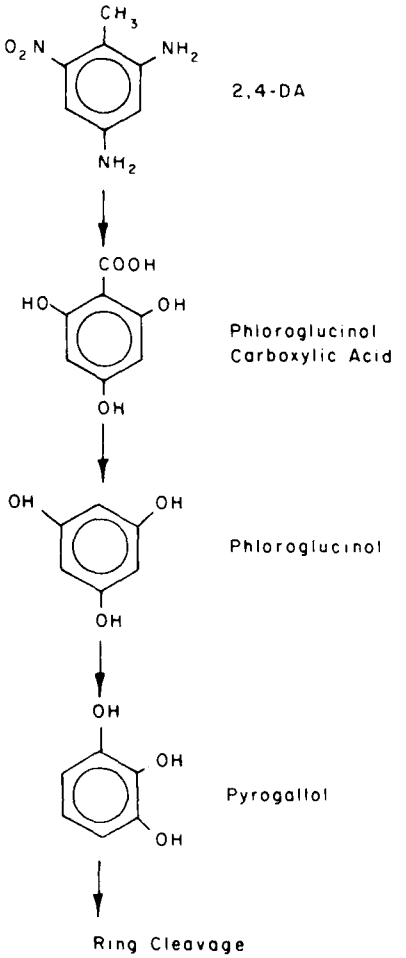

Figure 3. Pathway for 2,4-DA degradation (after Selivanovskaya et al. 1986).

rial cells, TNT and TNT degradation products sorbed onto the clay particles.

Naumova et al. (1988) compared the rates of TNT transformation by *Pseudomonas fluorescens* under conditions of oxygen and nitrate respiration. While TNT was reduced to 2-A and 4-A under both aerobic and anaerobic conditions, nitroreduction was somewhat faster for nitrate respiration. The subsequent reduction of these monoamino compounds to 2,4-DA was much more likely under nitrate respiration conditions. In contrast, degradation of 2,4-DA was faster under conditions of oxygen respiration. As a result, 2-A and 4-A predominated under aerobic conditions and 2,4-DA predominated under anaerobic conditions. When bacteria were grown on a medium with 2,4-DA as the sole source of nitrogen, phloroglucinol (1,3,5-THB) and pyrogallol (1,2,3-THB) were identified in the culture.

Two reports, published in Chinese with abstracts in English, describe TNT decomposition products in aqueous media. Xiong et al. (1986) treated TNT wastewater in an oxidative pond containing both algae and fungi. Biodegradation products included 4-A and 2-A. Lu et al. (1987) used UV to irradiate aqueous TNT solution (260–300 mg/L) at 50°C in the presence of ozone. After 3.5 hours, ~ 99% of the TNT was degraded and 2,3-dinitrophenylamine, 1,3-dinitrobenzene and 1,3,5-TNB were formed. Since the total organic carbon concentration decreased to less than 95 mg/L after 70 hours of irradiation, the authors suggest that TNT ring cleavage may have occurred with CO_2 evolution.

The aminodinitrotoluenes have also been identified as metabolic products of TNT in plants (Palazzo and Leggett 1986) and mammals (Yinon and Hwang 1986). Also, Palazzo and Leggett (1986) found that 2-A and 4-A were chemically bound to the plant material.

In summary, TNT is readily biotransformed in the environment to amino, diamino and azoxy compounds. Metabolites have been reported to polymerize and become tightly bound to organic materials. Some reports have been made of complete degradation of TNT to form CO_2, but the conditions under which this transformation took place were very specialized and not likely to be a major pathway in the environmental fate of TNT under natural conditions. Therefore, further analytical method development for the amino and diamino compounds is recommended. In the analyses of field-contaminated soils by CRREL's laboratory, 2-A and 4-A are often identified with 2-A predominating. In some samples, the sum of the amounts of 2-A and 4-A have actually exceeded the amount of TNT detected. Therefore, our experience leads us to believe that significant amounts of these intermediates may be detected in soils contaminated by TNT.

2,4-Dinitrotoluene (2,4-DNT)

McCormick et al. (1978) identified the products of biotransformation of 2,4-DNT by *Mucrosporium* sp. as 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 2,2′-dinitro-4,4′-azoxytoluene, 4,4′-dinitro-2,2′-azoxytoluene and 4-acetamide-2-nitrotoluene. The pathway proposed for this biotransformation is shown in Figure 4. Although 2,4-diaminotoluene was not detected, previous work with anaerobic bacteria demonstrated that both nitro groups can be enzymatically reduced to amino groups (McCormick et al. 1976), and that the 4-nitro group was reduced first.

Experiments at the Stanford Research Institute were conducted on the biodegradation of ^{14}C-2,4-DNT by mixed microbial populations. As reported by Isbister et al. (1980), 60% of the ^{14}C-label was evolved as $^{14}CO_2$ within 48 hours (how the DNT was labeled is not specified), and total degradation occurred within 1 week. Some amino derivatives and the condensation products were identified. The same microbial population did not yield $^{14}CO_2$ in experiments with ^{14}C-2,6-DNT.

Spanggord et al. (1980b) found similar results. Microorganisms from natural water bodies that could use 2,4-DNT as a sole source of carbon biotransformed more than 90% of the 2,4-DNT in 6 days. In studies with ring-labeled ^{14}C-2,4-DNT, 59% of the ^{14}C was evolved as $^{14}CO_2$ after 7 days of incubation. They identified one metabolite as 4-amino-2-nitrotoluene; nitroazoxytoluenes were also tentatively identified. These metabolites did not accumulate. The isomer 2,6-DNT was not degraded.

Liu et al. (1984) identified intermediates in the biotransformation of 2,4-DNT. They observed no degradation of 2,4-DNT after 14 days of incubation in aerobic fermenters. However, in anaerobic fermenters with methanol as the solvent carrier, 2,4-DNT was biotransformed and the metabolic products 2-nitroso-4-nitrotoluene, 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene accumulated and then disappeared. The nitroso compound was only detected between 48 and 72 hours of incubation, while the two amino compounds lasted almost the whole 14 days. In a subsequent experiment using a higher concentration of 2,4-DNT,

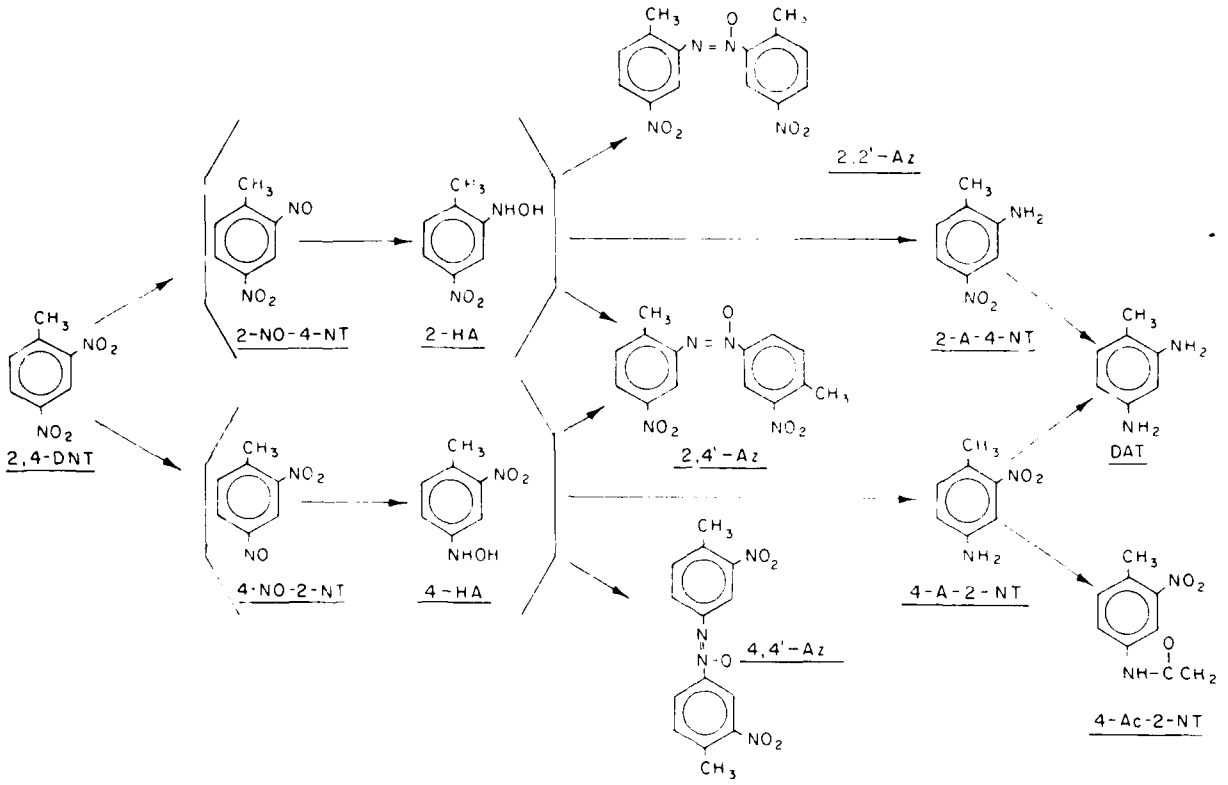

Figure 4. Pathway for 2,4-DNT (after McCormick et al. 1978).

they tentatively identified another intermediate as 4-nitroso-2-nitrotoluene. Couch et al. (1987) found that 2-amino-4-nitrotoluene and 2-nitroso-4-nitrotoluene were more mutagenic then 2,4-DNT itself when tested using a quantitative reversion assay with *Salmonella typhimurium* TA98.

Mori et al. (1984) found that the isomers of DNT were reduced via hydroxylaminonitrotoluenes to monoaminonitrotoluenes by *Escherichia coli* incubated aerobically for 28 hours. In an experiment with 2,4-DNT, the first metabolites detected were 4-hydroxylamino-2-nitrotoluene and 2-hydroxylamino-4-nitrotoluene; peak concentrations were reached at 8 hours. As the concentration of the hydroxylaminonitrotoluenes declined, there was a simultaneous increase in the concentrations of 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene. Other metabolic products such as the nitroso intermediates and diaminotoluene were not found.

In summary, the fate of 2,4-DNT in water bodies and soils has not been systematically defined, but the biotransformation of 2,4-DNT by cell cultures has been studied. Unlike TNT, 2,4-DNT may be completely metabolized to CO_2 by mixed microbial populations. Reduction products similar to those reported for TNT have also been found, namely the amino and azoxy derivatives. Presumably, these compounds would also be formed in soils. The development of analytical methods for the amino compounds is recommended, especially in light of their mutagenicity.

Trinitrobenzene (TNB) and 1,3-dinitrobenzene (DNB)

Like TNT and DNT, the principal mode of microbial transformation of the nitroaromatic compounds TNB and DNB is reduction of the nitro groups to form amino groups. McCormick et al. (1976) studied the reduction pathway of several nitroaromatic compounds including TNT, DNT, TNB and DNB in the presence of enzymes from *Veillonella alkalescens*. The pathway proceeded through a nitroso intermediate to hydroxylamino compounds. Further reduction yielded amino compounds.

Marino (1986) studied the reduction of the isomers of DNB using a variety of laboratory strains of plant, animal and microbial cells. Metabolites were confirmed by GC/MS to be the corresponding isomers of nitroaniline. Intermediates were

identified as the nitroso and hydroxylamine derivatives.

Evidence suggests that TNB is persistent in soils (Jerger 1976, Keirn et al. 1981) and in groundwater, while DNB is not. Incubation of DNB in water samples taken from the Tennessee River downstream from the Volunteer Army Ammunition Plant resulted in the microbially mediated conversion of the compound to CO_2. Mineralization of DNB did not occur in sterile controls or in water obtained from a local (Frederick, Maryland) source, indicating that the capability to degrade DNB by microorganisms will vary with the water source. In contrast, incubation of TNB in Tennessee River water did not result in biodegradation of the compound. A slow decline in TNB concentration was observed, and the transformation product 3,5,-dinitroaniline was identified (Mitchell and Dennis 1982, Mitchell et al. 1982). These results are consistent with our experience at CRREL, where hundreds of groundwater samples from present and former Army sites have been analyzed. TNB is routinely observed in those samples containing TNT. In some samples, the level of TNB exceeded that of TNT, 2-A and 4-A, while the levels of DNB are usually close to the level of detection.

NITRAMINES

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

The biodegradation of RDX has been studied under aerobic and anaerobic conditions. Several investigators have observed that RDX does not degrade under aerobic conditio . For example, Hoffsommer et al. (1978) found no bioconversion of RDX by aerobic-activated sludge microorganisms. Spanggord et al. (1980b) reported that RDX was persistent in aerobic water bodies. However, RDX degradation was significant under anaerobic conditions when extra organic nutrients were supplied. Soli (1973) reported a reduction in RDX concentration from 20 to 0.6 mg/L after 5 days in an anaerobic medium containing purple photosynthetic bacteria. Soli hypothesized that RDX was not metabolized; rather it was reduced during the photosynthetic process. McCormick et al. (1981, 1984a) monitored the degradation of RDX in nutrient broth cultures incubated anaerobically. As the concentration of RDX declined, there was an increase and sequential decrease of the mono-, di-, and trinitroso analogs of RDX formed by reduction of the nitro groups to nitroso groups. The anaerobic biodegradation pathway for RDX proposed by McCormick et al. (1981) is presented in Figure 5. The products include hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), hydrazine, 1,1-dimethylhydrazine, 1,2-dimethylhydrazine, formaldehyde and methanol. Upon subsequent exposure to aerobic conditions, the methanol may be metabolized to CO_2. The authors caution that the biodegradation products of RDX may pose more serious toxicological problems than RDX itself. Spalding and Fulton (1988) found RDX to be persistent in groundwater near Grand Island, Nebraska; none of the RDX degradation products mentioned above were found, even though some anaerobic conditions may have existed in some deep water wells.

Isbister et al. (1984) reported aerobic degradation in laboratory- and greenhouse-scale composting experiments using uniformly ring-labeled ^{14}C-RDX. After 6 weeks of composting, the solvent extractable RDX decreased to 21.6%. An average of 54.5% of the ^{14}C was evolved as CO_2, indicating cleavage of the RDX ring structure. The remaining ^{14}C in the solids was attributed to adsorbed RDX or breakdown products incorporated into the cell material. The leachates from the composts were nonmutagenic when tested using the Ames Assay.

Aerobic degradation of ^{14}C-RDX during composting was also reported by Doyle et al. (1986). For hay–horsefeed and sewage sludge composts, 46.5 and 37.2% of the ^{14}C added as ^{14}C-RDX was recovered as $^{14}CO_2$. Most of the remaining label (29.7 and 54.6%) was in residues that could not be extracted from the compost with acetonitrile using a 30-minute multiple extraction procedure. More RDX may have been extracted with a longer extraction time. Our studies at CRREL with field-contaminated soils have shown that at least 18 hours of extraction in a sonic bath is required to achieve equilibrium.

In the study conducted by Greene et al. (1985), in which simulated pink water was applied to soil columns, the mononitroso (MNX) and dinitroso (DNX) derivatives of RDX were identified in the leachate of the column with fast flow (100 mL/day) and carbon supplement (2.0 g/L glucose). MNX was also identified in the leachates from the columns with slow flow (40 mL/day) with and without carbon supplement.

RDX is susceptible to photodegradation. The photoproducts obtained when aqueous solutions of RDX were exposed to UV were reported by

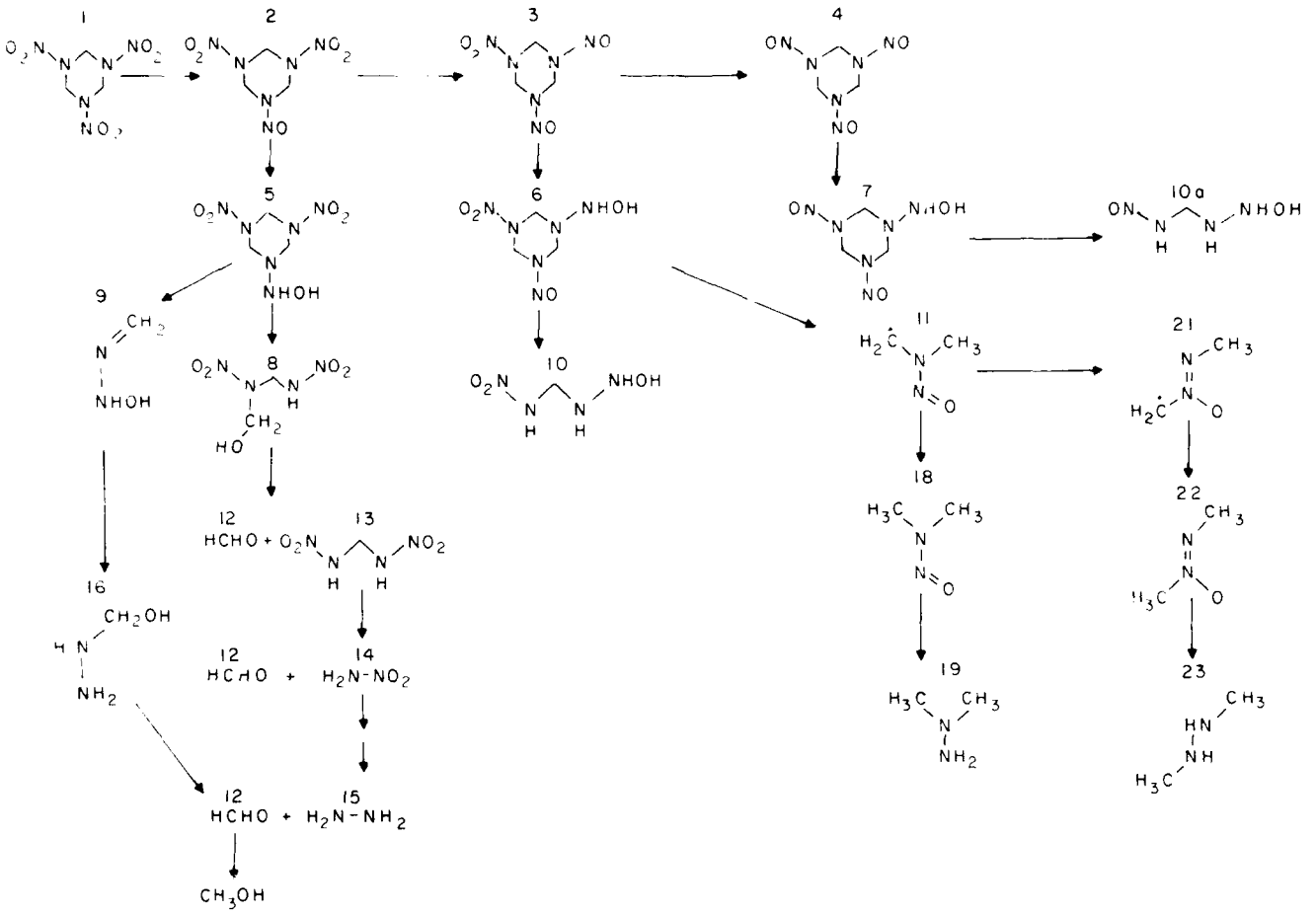

Figure 5. Pathway for RDX (after McCormick et al. 1981).

Glover and Hoffsommer (1979) to be nitrate, nitrite, ammonia, formaldehyde, nitrous oxide, formamide and N-nitroso-methylenediamine. The products of UV-O_3 produced CO_2, cyanic acid, nitrate, ammonia, and formic acid. Spanggord et al. (1980b) identified formaldehyde, nitrite and nitrate as photoproducts of RDX in water bodies.

In summary, RDX has been reported to be resistant to biodegradation under aerobic conditions. However, under anaerobic conditions with supplemental organic nutrients, RDX degrades substantially. Byproducts include methanol, formaldehyde, hydrazine and the nitroso-reduction products of RDX. RDX has also been reported to degrade in aerobic composts with evolution of CO_2. We may surmise that RDX may anaerobically transform in soils, groundwater and stagnant surface water, but the rate of biotransformation is unknown. Since the reduction mechanism proceeds through the nitroso derivatives (MNX, DNX and TNX) and nitroso derivatives were identified in soil column studies (Greene et al. 1985), analytical methodology is tentatively recommended for these compounds.

Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)

Only two reports were found that identified biodegradation products of HMX. Both Spanggord et al. (1983b) and McCormick et al. (1984b) studied the degradation of HMX under anaerobic conditions and detected the nitroso derivatives of HMX. Both studies described a pattern of peaks that appeared in chromatograms at various stages of HMX biotransformation (Fig. 6). As the peak area for HMX decreased, a second peak with an earlier elution time appeared, followed by a third peak with a still earlier elution time. GC/MS analysis indicated that these peaks were attributable to the mono, di- and in some cases trinitroso derivatives of HMX. The same pattern was observed for RDX, AcHMX and AcRDX (McCormick et al. 1984b), but

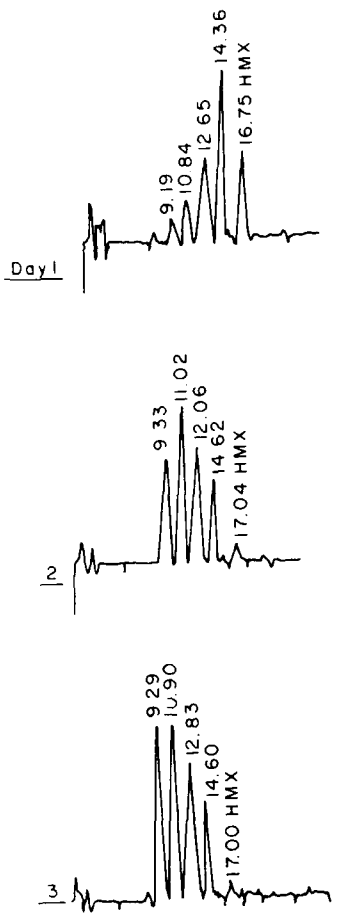

Figure 6. Chromatograms at various stages of HMX biotransformation (after McCormick 1983b).

the reaction rates for the eight-membered-ringed HMX and AcHMX were slower than for six-membered-ringed RDX and AcRDX. McCormick et al. (1984b) also detected methanol, which, they hypothesized, resulted from the reduction of formaldehyde, even though they did not detect formaldehyde.

In their soil column experiments, Greene et al. (1985) detected no transformation products of HMX in the column leachates. This result is consistent with the report of McCormick et al. (1984b) in that the eight-membered ring of HMX is less susceptible to biotransformation than the six-membered ring of RDX.

Spanggord et al. (1983b) also identified the photolytic products of HMX. The stable end products were nitrite, nitrate and formaldehyde (Fig. 7).

In the environment, HMX may undergo sequential reduction to form nitroso derivatives. The situation is similar to that of RDX; the potential for this transformation exists, but the relative amounts of reduction products are unknown.

Tetryl

No reports were found that identified biodegradation products of tetryl. However, thermal and photolytic products have been detected. Tamiri and Zitrin (1986), while conducting analyses for explosives by GC/MS, identified N-methylpicramide as a thermal degradation product of tetryl (Fig. 8). Kayser et al. (1984) reported picrate ion, N-methylpicramide, methylnitramine, nitrate and nitrite as photolytic products of tetryl in aqueous solution, and estimated the environmental hydrolysis half-life of tetryl to be 302 ± 76 days at 20°C and pH 6.8. Farey and Wilson (1975) also identified N-methylpicramide in tetryl samples

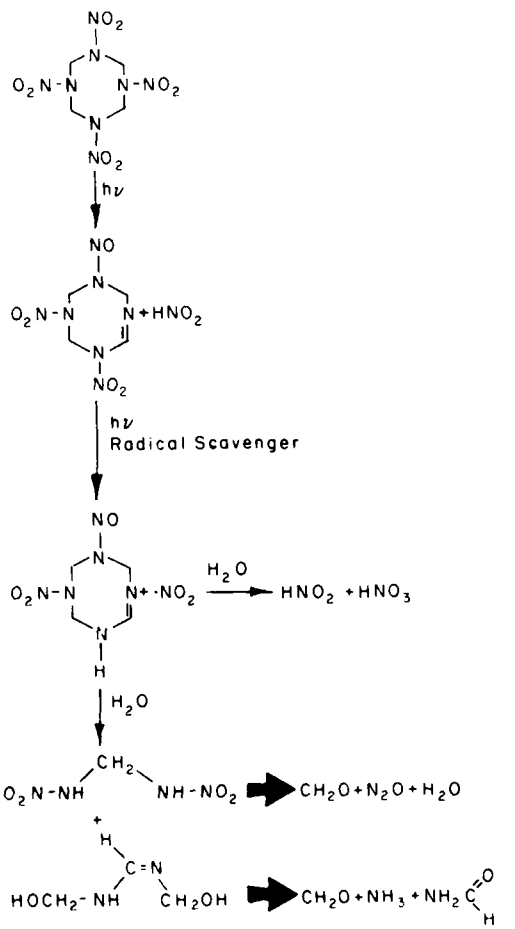

Figure 7. Photolytic products of HMX (after Spanggord 1983b).

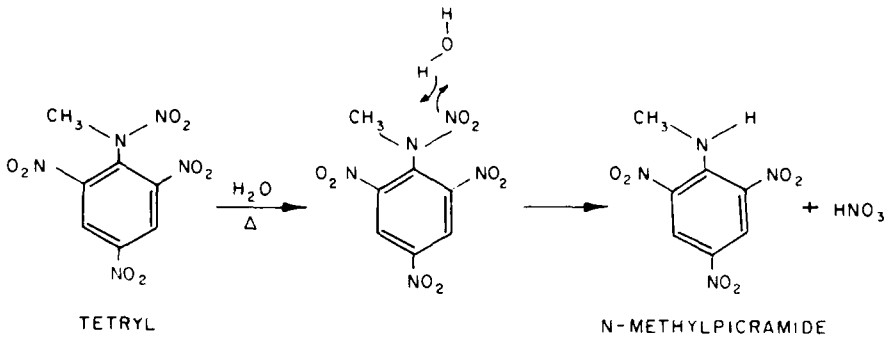

Figure 8. N-methyl picramide as a transformation product of tetryl (after Tamiri and Zitrin 1986).

heated under various conditions. These conditions included heating tetryl alone for 4 weeks at 80°C, alone for 4.5 days at 120°C and with lead azide for 4 weeks at 80°C. Other thermal degradation products found were picramide, picric acid, p-nitroaniline and trinitroanisole. Yasuda (1970) reported that production grade tetryl contains impurities such as N-methyl-N,2,4-trinitroaniline, picryl chloride, picric acid, picramide or N,2,4,6-tetranitroaniline and N-methyl-2,4,6-trinitroaniline. When tetryl samples were heated at 120°C for 48 hours, the major decomposition products were picric acid and 2,4,6-trinitroanisole. Smaller concentrations were also found of picramide or N,2,4,6-tetranitroaniline, N-methyl-2,4,6-trinitroaniline, 1,3,5-trinitrobenzene and N-methyl-N,2,4-trinitroaniline, or all five.

While developing an analytical method for nitroaromatics and nitramines in our laboratory, we found that tetryl thermally degraded while soils were extracted for 18 hours in a sonic bath at 45°C (Bauer et al. 1989). When chromatograms of soil extracts were examined (Fig. 9), we found a peak at 10.5 minutes for those samples in which tetryl had degraded. The information presented above suggests this product is probably N-methyl picramide.

REQUIREMENTS FOR ANALYTICAL METHODS

Chromatography has been used to detect and quantify the transformation products of nitroaromatics and nitramines. Appendix A contains some examples of parameters used for analysis and some sample chromatograms.

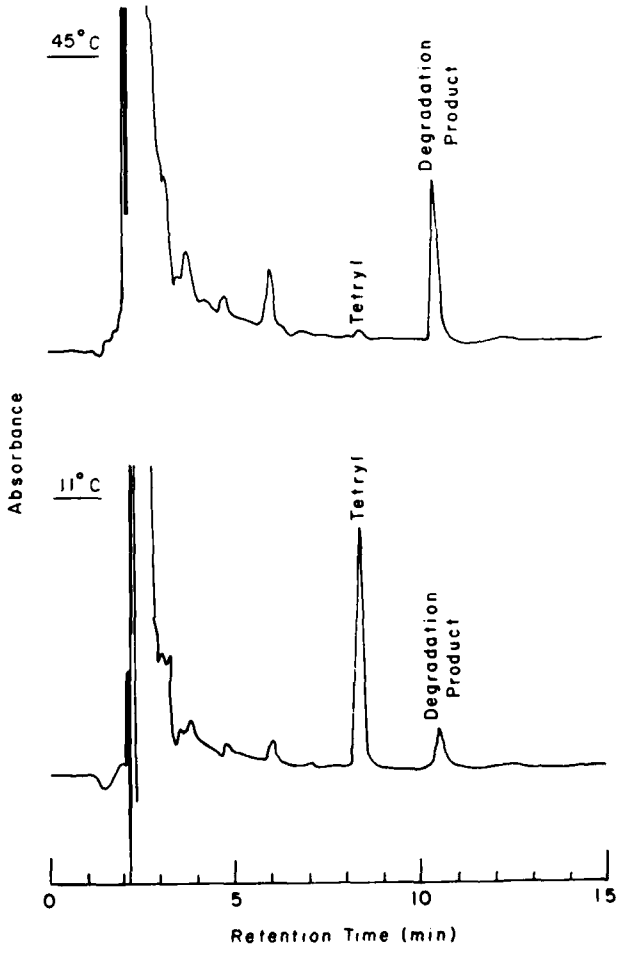

Figure 9. Chromatogram of tetryl degradation product.

When monitoring requirements are developed for these compounds, analytical methods will need to be certified at the appropriate contaminant levels. This certification procedure will require that analytical standards be made available. The stability of standards, both pure and in solution, must be clarified. Since most of the transformation products are transitory in the environment, sample handling methodology is of great importance and must be developed so that a true measure of analytes present at the time of sampling may be obtained. Standard practice for nitroaromatics and nitramines in soil and water is to store samples at 4°C for up to 1 week. Additional precautions may be needed when transition products are of interest (i.e., sterilization, protection from light, shorter storage times). As the standards and methods become available, in all likelihood, reports of transformation products in environmental samples will increase as more analysts gain the capability for detection and identification of these compounds.

As a first step in identifying those transformation products that may be present in munitions-contaminated soils, previously collected samples could be examined. CRREL has a large inventory of contaminated soils from Army sites from over ten states, and could use the samples to develop the necessary methodology.

CONCLUSIONS

Nitroaromatics and nitramines are susceptible to environmental transformation. Since some of the byproducts of this transformation may be persistent, and therefore of environmental concern, development of analytical standards and methodology is required to establish the prevalence of these compounds. Much research has been conducted on the environmental transformation of TNT. Since it is well established that 2-A, 4-A, 2,4-DA and 2,6-DA are frequently present in TNT contaminated matrices, certification of analytical methodology is highly recommended for these compounds. In addition, the presence of the coupling products 2,2'-Az, 2,4'-Az and 4,4'-Az should be validated. Since protocols for the liquid chromatographic analysis of TNT reduction products have been published, the availability of standards is the limiting factor for the validation.

The environmental fate of RDX is less defined than that of TNT. However, the nitroso derivative, MNX, has been detected in laboratory studies with soil columns. The presence of this compound in field-contaminated soil should be investigated. Analytical methodology is already established, but a source for standards is required.

Other compounds whose presence in the environment should be investigated include 2-amino-4-nitrotoluene, 4-amino-2-nitrotoluene, 3,5-dinitroaniline, 3-nitroaniline, 2-amino-4,6-dinitrobenzoic acid, DNX and TNX.

In conclusion, the immediate need is a source of standards so that environmental contamination may be validated. Next, certification of the analytical methods is required for those compounds deemed a true environmental concern. Established methods already exist for groups of compounds (primarily the source compound and reduction products). Since it is unlikely that a single analytical protocol will be adequate for all the byproducts that may be of interest, the most effective approach would be to emphasize the development of two separation schemes, one for nitroaromatic byproducts and one for nitramine byproducts.

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APPENDIX A: ANALYTICAL METHODS

Compounds		*Parameters*
TNT and reduction products in compost and soil extracts	Method:	RP-HPLC (Fig. A1)
	Column:	Waters μBondapak C18 (30 cm × 3.9 mm)
	Mobile phase:	Methanol–Water, gradient 40 → 78% MeOH, 2.5 mL/min
	Detection:	UV 230 nm
	Reference:	Kaplan and Kaplan (1982c)
TNT and reduction products in water	Method:	RP-HPLC (Fig. A2)
	Column:	Waters μBondapak C18 (30 cm × 3.9 mm)
	Mobile phase:	Methanol–Water, gradient 0 → 70% MeOH, 2.0 mL/min
	Detection:	UV 254 nm
	Reference:	Spanggord et al. (1980b)
TNT, 2-A, 4-A, 2,4-DA 2,6-DA, RDX	Method:	RP-HPLC (Fig.A3)
	Column:	Supelco C18 (3.3 cm × 4.6 mm) in line with Supelco CN (25 cm × 4.6 mm)
	Mobile phase:	Water–Methanol–THF 60.5:25:14.5 1.5 mL/min
	Detection:	UV 254 nm
	Reference:	Walsh and Jenkins (in prep.)
2-A in soil	Method:	RP-HPLC
	Column:	Supelco C18 (25 cm × 4.6 mm)
	Mobile phase:	Methanol–Water 1:1, 1.5 mL/min
	Detection:	UV 254 nm
	Reference:	Bauer et al. (1989)
2-A in water	Method:	RP-HPLC
	Column:	Supelco C3 (3.3 cm × 4.6 mm)
	Mobile phase:	Water–Methanol–THF 70.7:27.8:1.5 2 mL/min
	Detection:	UV 254 nm
	Reference:	Jenkins and Miyares (in prep)
HMX, RDX, 2-A, 4-A, TNT, TNB, Dinitroaniline in water	Method:	RP-HPLC (Fig. A4)
	Column:	Waters C18 Radial-Pak A
	Mobile phase:	Methanol–Acetonitrile–Water Gradient, 30 → 80%
	Detection:	UV 254 nm
	Reference:	Spanggord et al. (1983a)
2-amino-4,6-dinitrobenzoic acid, HMX, RDX, TNT, 2-A, 4-A in water	Method:	RP-HPLC (Fig. A5)
	Column:	Alltech C18 (25 cm × 4.6 mm)
	Mobile phase:	Gradient, 40 → 50% B in A A = 0.1M phosphate buffer (pH 7) with 0.05M TBAP; B = 0.0005M TBAP in methanol, 1.8 mL/min

	Detection:	UV 254 nm
	Reference:	Spanggord et al. (1983a)
TNT reduction products in compost extracts	Method:	GC-ECD
	Column:	2 mm i.d., 1.8-m glass column 1.5% PS2250/1.95% SP2401 on 100/120 Supelcoport
	Carrier:	Nitrogen, 28 mL/min
	Temp:	Port (210°C), Oven (180°C), Det (300°C)
	Reference:	Isbister et al. (1984)
TNT azoxy derivatives in compost extracts	Method:	RP-HPLC
	Column:	C18 (10 cm × 7.5 mm × 10 µm)
	Mobile phase:	70:30 Acetonitrile–Water 2 mL/min
	Detection:	UV 232 nm
	Reference:	Isbister et al. (1984)
DNT reduction products in culture broths	Method:	GC (Fig. A6)
	Column:	Chromosorb W(AW-DMCS), 3% OV-1 (2 mm × 180 cm)
	Oven temp:	130 → 210°C (4°C/min)
	Detection:	FID or ECD
	Reference:	Liu et al. (1984)
Nitroso derivatives of RDX in culture medium	Method:	RP-HPLC
	Column:	Waters µBondapak C18 (30 cm × 3.9 mm)
	Mobile phase:	20:80 Methanol–Water, 2.5 mL/min
	Detection:	UV 230 nm
	Reference:	McCormick et al. (1984a)
Nitroso derivatives of HMX in culture medium	Method:	RP-HPLC (Fig. A7)
	Column:	Waters µBondapak C18 (30 cm × 3.9 mm)
	Mobile phase:	20:80 Acetonitrile–Water 2.5 mL/min
	Detection:	UV 230 nm
	Reference:	McCormick et al. (1984a)
Hydrolysis products of tetryl	Method:	Ion-pairing RP-HPLC (Fig. A8)
	Column:	Hewlett Packard C8 (25 cm × 4.6 mm × 10 µm)
	Mobile phase:	50:50 Methanol–Water, 0.005 M tetrabutyl ammonium phosphate, pH 7.5 (Pic A reagent)
	Detection:	UV 254 nm
	Reference:	Kayser et al. (1984)

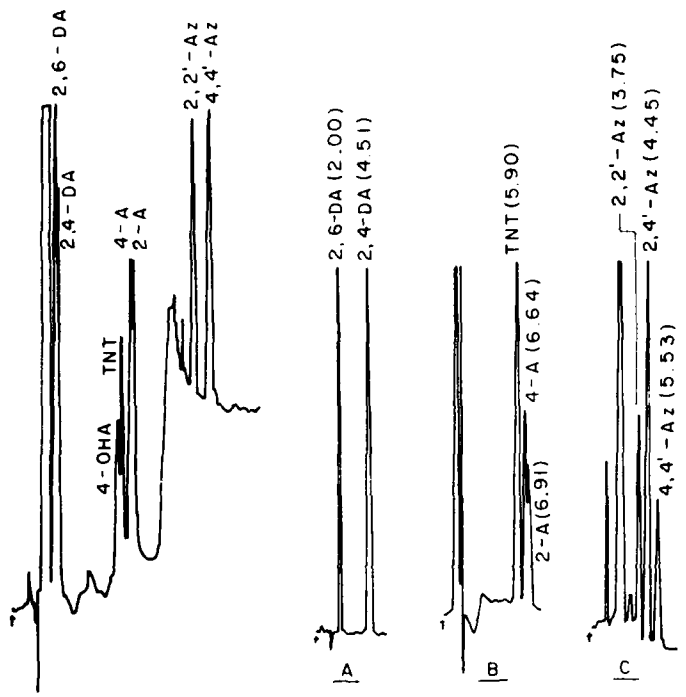

Figure A1. Separation of TNT reduction products (after Kaplan and Kaplan 1982c).

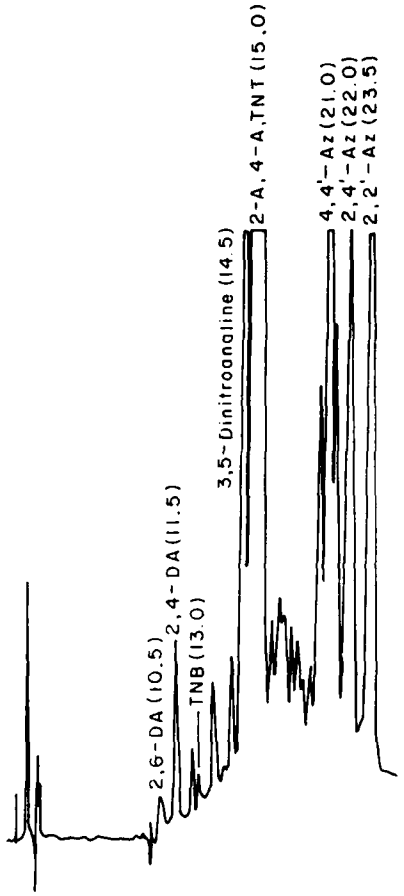

Figure A2. Separation of TNT reduction products (after Spanggord et al. 1980b).

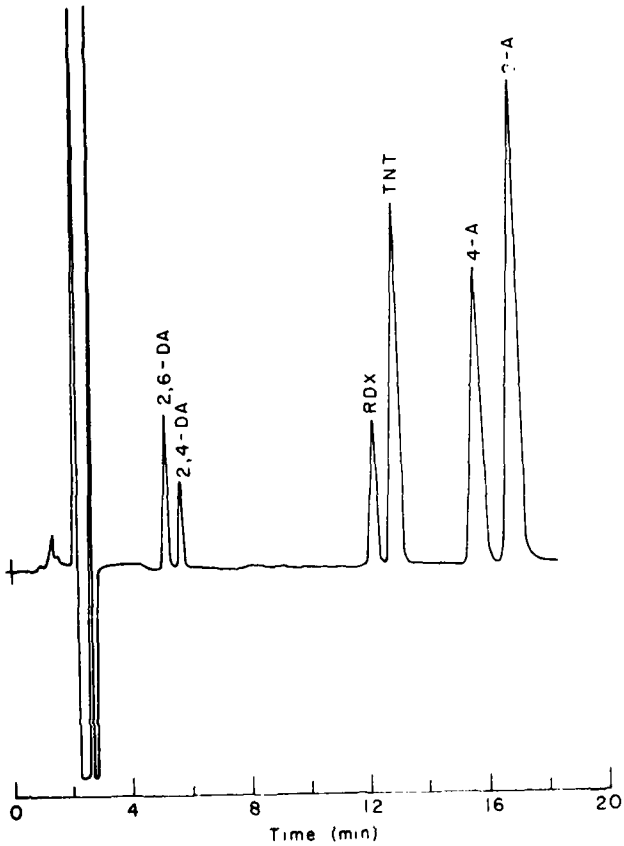

Figure A3. Separation of TNT reduction products.

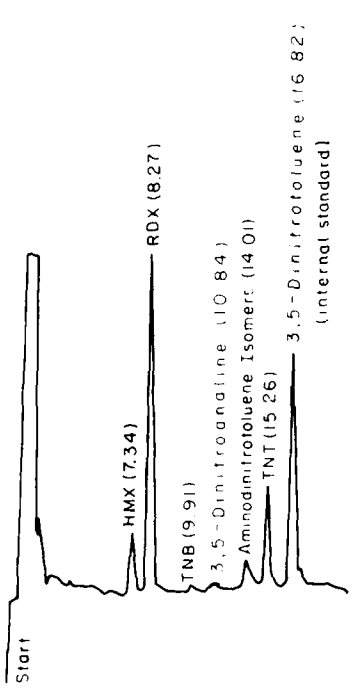

Figure A4. Chromatogram of lagoon water from Louisiana Army Ammunition Plant (after Spanggord et al. 1983a).

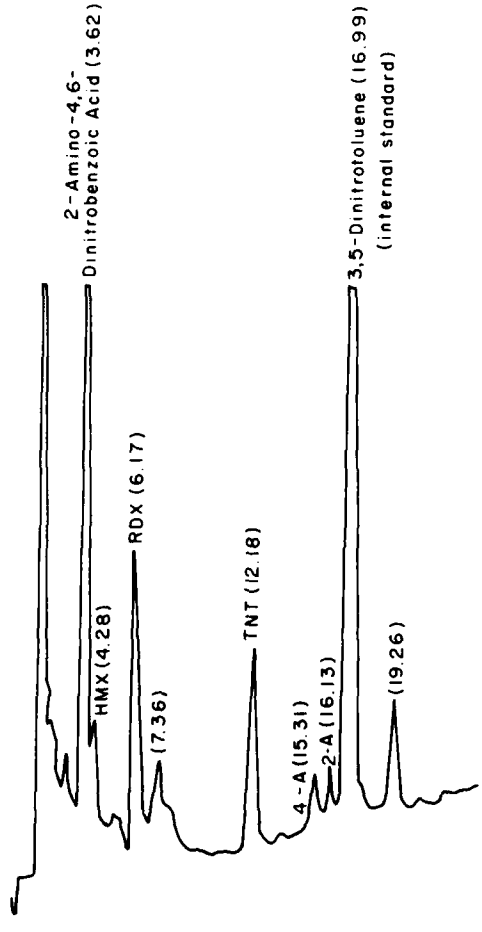

Figure A5. Chromatogram of lagoon water from Louisiana Army Ammunition Plant showing resolution of 2-amino-4,6-dinitrobenzoic acid from other wastewater components (after Spanggord et al. 1983a).

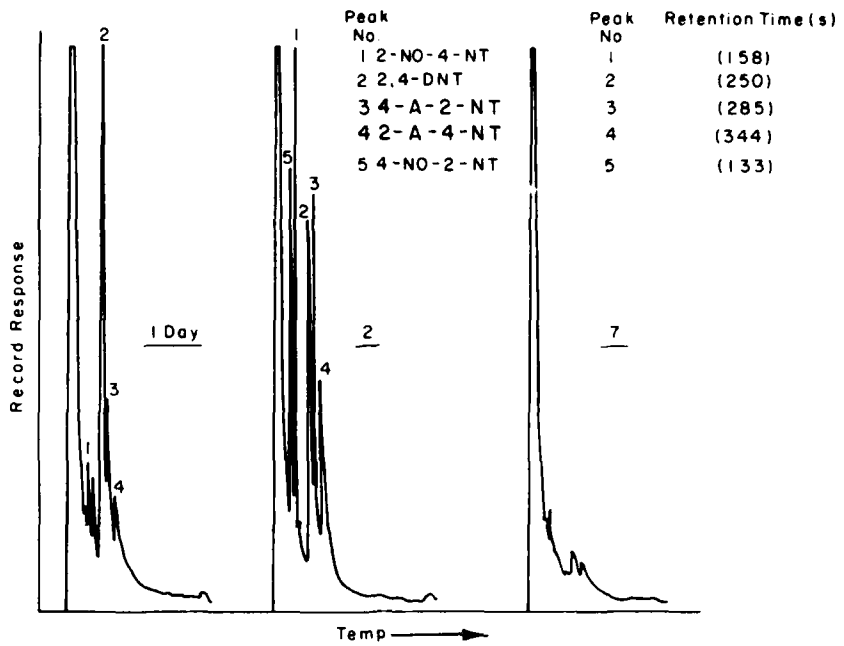

Figure A6. Gas chromatograms of extracts from an anaerobic fermenter containing 2,4-DNT (after Liu et al. 1984).

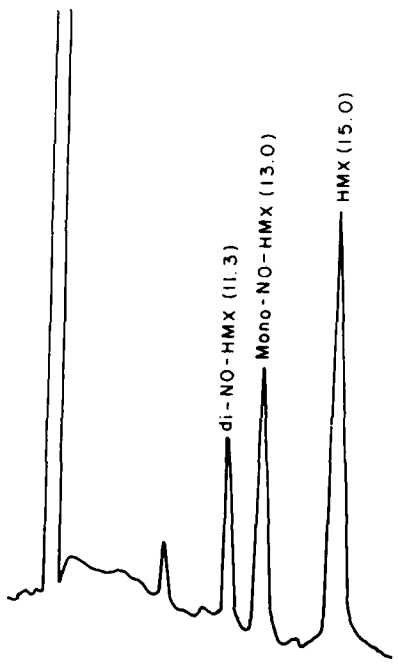

Figure A7. Separation of nitroso derivatives of HMX (after McCormick et al. 1984a).

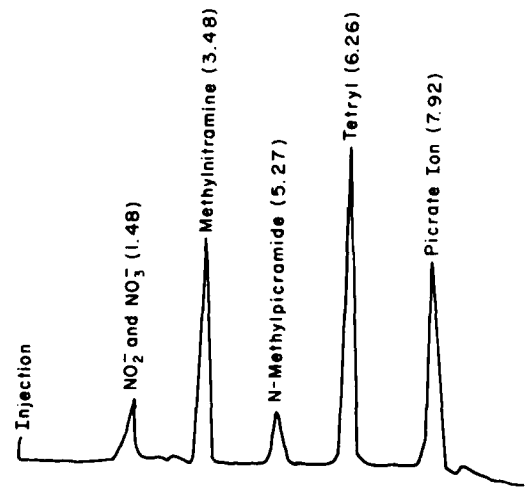

Figure A8. Separation of tetryl hydrolysis products (after Kayser et al. 1984).

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13. ABSTRACT (*Maximum 200 words*)

The literature describing the environmental transformation of organic explosives and related compounds is reviewed in an attempt to identify those byproducts for which certified analytical methods should be developed. Among those compounds identified are TNT reduction products (aminodinitrotoluenes and diaminonitrotoluenes) and coupling products (azoxytoluenes). The development of methods is also recommended for the amino derivatives of DNT, TNB and DNB, as well as the nitroso derivatives of HMX and RDX.

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